REMARKS

Claims 1-14 and 17-34 were pending in the instant application. Claims 1-13 and 23-34 have been withdrawn from consideration as being directed to non-elected subject matter. Claims 1-13 and 23-34 are cancelled by the instant amendment, without prejudice. Applicants reserve the right to pursue the non-elected subject matter in this or a separate application(s). Claims 14 and 17-19 have been amended and claim 35 has been added. The claim amendments and addition are intended to more clearly indicate the claimed subject matter. Support for the claim amendments and newly added claim can be found throughout the specification and claims as originally filed. Upon entry of the instant amendment, claims 14, 17-22 and 35 will be pending.

No new matter has been added to the application. APPENDIX A includes a marked-up version of amended claims 14 and 17-19 and amended paragraphs of the specification. All pending claims, whether amended or not, are set forth for the Examiner's convenience as APPENDIX B.

The foregoing claim amendments and/or cancellations were made solely to expedite prosecution. Applicants reserve the right to pursue the same or similar subject matter as encompassed by the amended and/or cancelled claims herein or as originally filed in this or a separate application(s).

Objection to Drawings

The drawings filed on August 12, 2002 have been objected to as allegedly introducing new matter. The objection appears to be based on the recitation of the sequence identifier "SEQ ID NO:13" in Figure 4B. The recitation of "SEQ ID NO:13" is alleged to be "new matter". Applicants' submit that the recitation of sequence identifiers in Figure 4B is not "new matter". Rather the recitation of sequence identifiers is a requirement for patent applications including nucleotide and/or amino acid sequences.

The sequence identifier "SEQ ID NO:13" has been assigned by Applicants to the portion of RDE-1 depicted in Figure 4B. The full length sequence for RDE-1 is set forth as SEQ ID NO:1. The partial RDE-1 amino acid sequence is clearly supported in the application (informal Figure 4B) as-filed and has merely been assigned a new sequence identifier in accordance with sequence listing rules. As the incorporation of sequence identifiers does not amount to "new matter", Applicants respectfully request withdrawal of the objection.

Objection to Specification

The amendment filed on August 12, 2002 has been objected to as introducing new matter, in particular, based on a substitute sequence listing submitted with the amendment. A replacement Sequence Listing (computer-readable form and paper copy) is in preparation and will be submitted imminently. The following table sets for the sequences included in the replacement sequence listing as well as an indication as to where support for each sequence can be found in the application as originally filed.

SEQ ID NO:	**Sequence**	**Support**
1	*rde-1* genomic sequence	Figure 5A-C
2	*rde-1* cDNA sequence	Figure 6A-D
3	RDE-1 amino acid sequence	Figure 6A-D
4	*rde-4* cDNA sequence	Figure 10A-B
5	RDE-4 amino acid sequence	Figure 10A-B
6	ZWILLE (regions of homology with RDE-1)	Figure 4B
7	Sting (regions of homology with RDE-1)	Figure 4B
8	consensus sequence	Figure 11
9	F48F7.1 (regions of homology with RDE-1)	Figure 4B

10	eIF2C (regions of homology with RDE-1)	Figure 4B
11	X1RBPA (regions of homology with RDE-4)	Figure 11
12	HsPKR (regions of homology with RDE-4)	Figure 11
13	RDE-1 (internal portion)	Figure 4B
14	RDE-4 (internal portion)	Figure 11

No new matter has been added. Applicants respectfully request withdrawal of the objection to the specification.

Acknowledgment of Claims Free of Prior Art

Applicants gratefully acknowledge that the Examiner, in paragraph 9 of the instant Office Action, has indicated claims 14 and 17-22 free of the art *i.e.*, no references found teaching or suggesting claims 14 and 17-22.

Rejection of Claims 14 and 17-22 Under 35 U.S.C. § 112, First Paragraph

Claims 14 and 17-22 stand rejected under 35 U.S.C. §112, first paragraph, as being non-enabled. The Examiner states that the specification, while being enabled for effecting RNAi in the cells of *C. elegans*, does not reasonably provide enablement for effecting RNAi in the cell of any other organism. In particular, the Examiner states that the specification does not enable the skilled artisan "to make and use the invention commensurate in scope with the claims". Applicants traverse.

The Examiner looks to several references published prior to the filing of the instant application and states that while the references teach the conserved phenomenon of gene silencing in organisms ranging from plants to flies to fungi, the references do not teach the individual components of the RNAi pathways or identify components essential

to the RNAi pathways in the various systems described. The Examiner concludes that the skilled artisan would have to perform undue experimentation to identify RNAi components in these systems in order to practice the instant invention in other organisms. The Examiner fails to appreciate, however, that the skilled artisan does not need to experimentally identify the components essential to the RNAi pathway in other systems but rather can rely on the teachings of the instant application to select RNAi components from other organisms suitable for use in preparing RNAi agents.

An essential teaching of the instant invention is that dsRNA-induced gene silencing operates *via* mechanisms that are conserved among a variety of distinct organisms including plants, animals and fungi. Having identified important components of the RNAi machinery in *C. elegans*, the specification further teaches how to identify, obtain and/or select similar reagents from other systems for use in the claimed methodologies. The specification teaches, for example, the isolation of homologous genes using two-hybrid screens, hybridization-based assays, complementation-based assays, PCR, and/or database screens, (see, for example, page 10, line 4 through page 12, line 6) all of which are facilitated by the disclosure of the sequences of the *C. elegans* RNAi components, *e.g.*, RDE-1 and/or RDE-4

The instant specification teaches that the RNAi components identified in *C. elegans*, *e.g.*, RDE-1 and RDE-4, belong to conserved gene families and, moreover, teaches other family members which share a significant degree of sequence identity and/or structural features with the *C. elegans* genes. The specification teaches, for example, that RDE-1 share a significant degree of sequence identity and/or structural features with the Zwille and argonaute proteins from *Arabidopsis*, the Sting and Piwi proteins from *Drosophila*, the eIF2C protein from rabbit, as well as others (see Figure 4B and Example 6). These RDE-1 homologues share significant identity (as depicted, for example, in the sequence alignment of Figure 4B) and are most highly conserved across

their Paz and Piwi domains. The specification also teaches proteins having homology to RDE-4, in particular, across the DNA-binding domain (see *e.g.*, Figure 11 and Example 11).

Thus, the instant specification is replete with teachings of RNAi components from other systems for use according to the claimed methodologies. These components were identified by the instant inventors using database screens for sequences homologous to the RNAi pathway genes identified by genetic screening. References published after Applicants' filing date are discussed herein which reinforce Applicants' teaching that these homologues are functionally related, in addition to their structural relatedness. (Abstracts or front pages of the references discussed are included for the Examiner's instant convenience. Full copies of the references are being included in a Supplemental Information Disclosure Statement to be submitted imminently).

For example, the Fagard *et al.* reference presents data demonstrating that the *Arabidopsis* protein, Argonaute 1 ("AGO1"), is required for gene silencing in plants. The Williams and Rubin reference further demonstrates the activity of AGO1 in mediating RNAi in *Drosophila*. Both the Fagard *et al.* reference and Catalanotto *et al.* reference, teach that the *N. crassa* protein QDE-2, which shares both sequence identity and conserved structural domains with RDE-1 and Argonaute-1 (see *e.g.*, Figure 1 of each reference) controls gene silencing in fungi. The Pal-Bhadra *et al.* reference demonstrates that the *Drosophila* protein, Piwi, is required for gene silencing in flies. Lastly, the Doi *et. al.* reference demonstrates a role for eif2C in promoting RNAi in a mammalian system.

The instant specification teaches methods for identifying RNAi pathway genes, in particular, homologues of the *C. elegans* RDE-1 and RDE-4 genes identified by Applicants. References published shortly after Applicants' filing date evidence that these homologues are required for and/or useful in promoting RNAi in a variety of systems.

The skilled artisan, seeking to practice the invention, would find ample teachings in the instant specification as to RNAi pathway components from other systems useful for making RNAi agents. Moreover, RNAi agents made using *C. elegans* RNAi pathway components could be used for effecting RNAi in other systems. The Examiner makes reference to "an added complication arising in mammalian cells", namely the PKR response, and indicates that overcoming this response would add to the experimentation necessary to practice the claimed invention in organisms other than *C. elegans*. Applicants submit, however, that at the time the instant application was filed, numerous methods for overcoming the PKR response were known to the skilled artisan. For example, peptide antagonists of PKR were known, see *e.g.*, the Judware and Petryshyn reference, the Nekhai *et al.* reference and WO 98/04717. Antisense oligonucleotides effective at inhibiting PKR were also known, see *e.g.*, the Maitra *et al.* reference and WO98/54315. Moreover, overcoming the PKR response is not necessary in all mammalian systems. For example, the Svoboda *et al.*, Wianny and Zernicka-Goetz and Billy *et al.* references demonstrate that RNAi can be effected by dsRNA in mouse oocytes or embryonic teratocarcimona cell lines, without a concomitant PKR response.

Based on the substantial teachings in the instant specification as to RNAi components suitable for use in making RNAi agents, as well as knowledge in the art regarding overcoming the PKR response certain systems, *e.g.*, mammalian systems, any experimentation required by the skilled artisan to practice the invention within the scope of the claims would be routine, rather than undue. In view of the above, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 14 and 17-22 under 35 U.S.C. §112, first paragraph.

CONCLUSION

In view of the above amendments and remarks, it is believed that this application is in condition for allowance. If a telephone conversation with Applicants' Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 227-7400.

Date: May 15, 2003

LAHIVE & COCKFIELD, LLP
Attorneys at Law

By _____
Debra J. Milasincic, Esq.
Reg. No. 46,931
28 State Street
Boston, MA 02109
(617) 227-7400
(617) 742-4214

APPENDIX A
Marked-up Version of Claims to Show Changes

Claims 14 and 17-19 have been amended, as follows:

14. (Twice amended) A method of inhibiting the activity of a gene, the method comprising introducing an RNAi agent into a cell, wherein the RNAi agent [comprises] is prepared by incubating a double-stranded RNA (dsRNA) component in the presence of an RNAi pathway component, and wherein the dsRNA component is targeted to the gene.

17. (Amended) The method of claim 14, wherein the RNAi [agent is prepared by incubating a double-stranded RNA in the presence of] pathway component is an RDE-1 polypeptide.

18. (Amended) The method of claim 14, wherein the RNAi [agent is prepared by incubating a double-stranded RNA in the presence of] pathway component is an RDE-4 polypeptide.

19. (Amended) The method of claim [14] 35, wherein the [RNAi agent is prepared by incubating a double-stranded RNA in the presence of] RNAi pathway components are an RDE-1 polypeptide and an RDE-4 polypeptide.

The specification has been amended as follows:

The paragraph beginning at page 7, line 30 (spanning pages 7-8), has been replaced with the following re-written paragraph:

Figure 4B is [an illustration of] a depiction of regions of homology between the predicted sequence of RDE-1 and [its alignment with] four related proteins. The sequences are RDE- 1 (*C elegans*; Genbank Accession No. AF180730) (SEQ ID NO:13), F48F7.1 (*C elegans*; Genbank Accession No. Z69661) (SEQ ID NO:9), eIF2C (rabbit; Genbank Accession No. AF005355) (SEQ ID NO: 10), ZWILLE (*Arabidopsis*; Genbank Accession No. AJ223508) (SEQ ID NO:6), and Sting (*Drosophila*; Genbank Accession No. AF145680) (SEQ ID NO:7). Identities with RDE- 1 are shaded in black, and identities among the homologs are shaded in gray.

The paragraph beginning at page 9, line 6, has been replaced with the following re-written paragraph:

Figure 11 is a depiction of regions of homology between the predicted RDE-4 amino acid sequence (SEQ ID NO:14), X1RBPA (SEQ ID NO:[6] 11), HsPKR (SEQ ID NO:[7] 12), and a consensus sequence (SEQ ID NO:8). A predicted secondary structure for RDE-4 is also shown illustrating predicted regions of α helix and β pleated sheet.

The paragraph beginning at page 47, line 14, has been replaced with the following re-written paragraph:

Analysis of the *rde-4* nucleic acid sequence shows that it encodes a protein (RDE-4) with similarities to dsRNA binding proteins. Examples of the homology to X1RBPA (SEQ ID NO:[6] 11; Swissprot: locus_TRBP_XENLA, accession Q91836; Eckmann and Jantsch, 1997, J. Cell Biol. 138:239-253) and HSPKR (SEQ ID NO:[7] 12; AAF13156.1; Xu and Williams, 1998, J. Interferon Cytokine Res. 18:609-616), and a consensus sequence (SEQ ID NO:8) are shown in Fig. 11. Three regions have been identified within the predicted RDE-4 protein corresponding to conserved regions found in all members of this dsRNA binding domain family. These regions appear to be important for proper folding of the dsRNA binding domain. Conserved amino acid residues, important for interactions with the backbone of the dsRNA helix, are found in all members of the protein family including RDE-4 (see consensus residues in Figure 11). This motif is thought to provide for general non-sequence-specific interactions with

dsRNA. The RDE-4 protein contains conserved protein folds that are thought to be important for the assembly of the dsRNA binding domain in this family of proteins. Conserved amino acid residues in RDE-4 are identical to those that form contacts with the dsRNA in the crystal structure of the XlRBP dsRNA complex. These findings strongly suggest that RDE-4 is likely to have dsRNA binding activity.

APPENDIX B
Pending Claims

14. (Twice amended) A method of inhibiting the activity of a gene, the method comprising introducing an RNAi agent into a cell, wherein the RNAi agent is prepared by incubating a double-stranded RNA (dsRNA) component in the presence of an RNAi pathway component, and wherein the dsRNA component is targeted to the gene.

17. (Amended) The method of claim 14, wherein the RNAi pathway component is an RDE-1 polypeptide.

18. (Amended) The method of claim 14, wherein the RNAi pathway component is an RDE-4 polypeptide.

19. (Amended) The method of claim 35, wherein the RNAi pathway components are an RDE-1 polypeptide and an RDE-4 polypeptide.

20. The method of claim 14, wherein the RNAi agent is introduced into the cell in a liposome.

21. The method of claim 14, wherein the RNAi agent is introduced into the cell by injection.

22. The method of claim 14, wherein the cell is in an animal.

35. (New) The method of claim 14, wherein the RNAi agent is prepared by incubating the dsRNA component in the presence of at least two RNAi pathway components.